Absence of Association Between Serum Homocysteine Levels and Coronary Artery Disease in South Indian Males

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Background: Asian Indians are reported to have a very high prevalence of premature coronary artery disease. However, traditional risk factors do not explain this excess of coronary artery disease. Elevated levels of homocysteine are reported to be associated with coronary artery disease among Europeans. This study looked at the association of serum homocysteine levels with coronary artery disease in South Indians.

Methods and Results: Four groups of patients were studied: Group 1 consisted of healthy nondiabetic subjects without coronary artery disease (n=18); Group 2 consisted of nondiabetic subjects with coronary artery disease (n=21); Group 3 consisted of type 2 diabetic patients without coronary artery disease (n=18) and Group 4 consisted of type 2 diabetic patients with coronary artery disease (n=20). The mean homocysteine value was 12.4±3.4 µmol/L in Group 1; 12.6±4.6 µmol/L in Group 2; 10.1±4.4 µmol/L in Group 3; and 10.4±3.9 µmol/L in Group 4. There was no significant difference in the homocysteine levels between the groups studied. The prevalence of hyperhomocysteinemia, defined as a level of 17.1 µmol/L (the 95th percentile for serum homocysteine in the control group) was not significantly different among the groups.

Conclusions: Elevated serum homocysteine levels are not associated with coronary artery disease in South Indian male subjects with or without diabetes. However, the results must be interpreted with caution because of the small numbers studied. (Indian Heart J 2001; 53: 44-47)

Key words: Hyperhomocysteinemia, Coronary disease, Risk factors

Hyperhomocysteine, a thiol containing amino acid generated during the metabolism of methionine, occurs in almost all human tissues. Elevated levels of homocysteine are associated with vascular disease and hyperhomocysteinemia is thus considered to be an independent risk factor for coronary artery disease (CAD). Factors which influence the levels of homocysteine include age, genetics, and nutrition. Since genetic background and nutritional intake vary in different populations, studies on homocysteine levels in different ethnic groups are necessary.

Asian Indians have very high prevalence rates of premature CAD occurring below the age of 50 years. Conventional risk factors do not fully explain the excess of CAD among Indians, hence the need for studying newer cardiovascular risk factors. The increased susceptibility to CAD in this ethnic group suggests that genetic factors may play a role. As homocysteine levels are genetically determined, we undertook a study of homocysteine levels in South Indian diabetic and nondiabetic subjects with and without CAD in Tamil Nadu state (South-East India).

Methods

Study Groups: We restricted the study to males as we could not obtain a sufficient number of well-characterized female subjects. The following groups of subjects were studied.

Group 1 consisted of 18 healthy nondiabetic control subjects, selected from an ongoing population-based study by the Chennai Urban Population Study (CUPS), Chennai. The inclusion criteria were: normal glucose tolerance test, absence of angina, myocardial infarction or any history of vascular disease and a normal resting 12-lead electrocardiogram (ECG).

Group 2 consisted of nondiabetic patients with CAD. Twenty-one male patients were selected from the department of Cardiothoracic Surgery, Government General Hospital, Chennai. CAD was diagnosed on the basis of...

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of coronary angiography and all the subjects had severe (>70%) stenosis of two or more coronary arteries.

Groups 3 and 4 were type 2 diabetic patients selected from the MV Diabetes Specialities Centre, a tertiary diabetes care center at Chennai. The diagnosis of diabetes was based on past medical history, drug treatment for diabetes (insulin or oral hypoglycemic agents) and/or criteria laid down by the WHO Consultation Report, i.e. fasting plasma glucose ≥126 mg/dl and/or 2 hour plasma glucose ≥200 mg/dl.

Group 3 consisted of 18 type 2 diabetic patients without CAD. All patients in this group denied any history of angina or myocardial infarction, had no history of any vascular disease and had normal resting 12-lead ECGs.

Group 4 consisted of 20 type 2 diabetic patients with CAD, which was diagnosed angiographically using criteria similar to those for Group 2.

The study protocol was approved by the ethical committee of our center and informed consent was obtained from all the study subjects. Clinical examination included measurement of height, weight, calculation of body mass index, blood pressure measurement and cardiovascular examination.

**Biochemical Methods:** A fasting blood sample was taken from all the study subjects for the estimation of plasma glucose, serum lipids and homocysteine. Homocysteine was assayed by enzyme-linked immunoassay (ELISA) using an Axis Biochemicals kit (Bio Rad, CA, USA). Briefly, protein-bound homocysteine in the serum sample is reduced to free homocysteine and converted enzymatically to S-adenosyl homocysteine (SAH) before the immunoassay. This is followed by a solid-phase enzyme immunoassay based on competition between SAH in the sample and immobilized SAH bound to the wall of the microtiter plate for binding sites on a monoclonal anti-SAH antibody. After removal of the unbound anti-SAH antibody, a secondary rabbit anti-mouse antibody labeled with the enzyme horseradish peroxidase is added. The peroxidase activity is measured spectrophotometrically after addition of the substrate. The absorbance is inversely related to the concentration of total homocysteine in the sample. A calibration curve is constructed using a standard (2.0 – 50.0 µmol/dl) provided with the kit. The inter- and intra-assay coefficient of variation of the homocysteine assay was 8.3% and 9.8%, respectively. Total serum cholesterol, serum triglyceride and HDL cholesterol were assayed with a commercial kit (Boehringer Mannheim, Germany) using the Hitachi 912 Autoanalyser (Boehringer Mannheim, Germany). LDL was calculated using the Friedewald equation.

Hypertension was diagnosed based on a history of drug treatment or if the blood pressure was greater than 140/90 mmHg.

**Statistical analysis:** One-way ANOVA was used to compare the mean of the continuous variables among the study groups. Chi-square test or Fisher's exact test were used as appropriate to compare the proportions. All analyses were performed with the SPSS statistical software package (Version 4.0.1. SPSS, Chicago) and p values <0.05 were considered significant.

**Results**

Table 1 shows the clinical and biochemical features of the study population. Both the diabetic and nondiabetic patients with CAD had a higher prevalence of hypertension and smoking. Both the diabetic groups had higher serum cholesterol and serum triglyceride levels compared to the respective nondiabetic groups.

There was no difference in the mean homocysteine levels in the subjects, with or without CAD, either in the diabetic or in the nondiabetic subjects. The 95th percentile for serum homocysteine in the control group was 17.1 µmol/L. Using this as the definition, the prevalence of hyperhomocysteinemia was not significantly different among the groups.

There was a wide scatter of the values in the distribution of homocysteine in the study groups as seen in Fig. 1.
Discussion

Recent reports on homocysteine suggest that it is an independent predictor of vascular disease, including stroke and CAD.3–6 A recent study has demonstrated the association of plasma homocysteine with increased CAD among immigrant Indians in the UK.19 However, a study from Singapore showed no increase in homocysteine levels in Indians compared to the Malays and Chinese.20 There are very few studies on homocysteine levels in native Indians. The present study shows a lack of association of homocysteine with CAD in native Indians. Similar results were obtained in a study from Kerala in South-West India.21 However, these results must be interpreted with caution in view of the limitations of the study mentioned below.

Studies on the association of hyperhomocysteinemia with CAD in different populations have yielded conflicting results, with some studies providing evidence for an association19,22,23 while others have found none.21,24,25 This may be attributed to ethnic differences or due to differences in the definition of cases or sample sizes.

Recent studies on homocysteine in Indian diabetic patients have revealed an association of body weight with plasma homocysteine levels.26 The study by Munshi et al.27 showed elevated homocysteine levels in diabetic subjects compared to age-matched controls. Hoffman et al.28 reported that hyperhomocysteinemia is more common in type 1 diabetic patients with nephropathy. It was recently demonstrated that insulin may play a role in homocysteine metabolism29 and obese type 2 diabetics tend to have higher levels of homocysteine due to hyperinsulinemia.26 Our data show that there was no significant increase of homocysteine levels in diabetes per se or in diabetic patients with CAD. This could be attributed to the fact that the body-mass index was similar among the study groups.

One of the reasons for the discrepancy in results obtained from studies carried out on Europeans and Indians may be due to the differences in dietary habits. In India, many people are vegetarians and there is a higher intake of green leafy vegetables that are rich in folate which is an essential co-factor in the metabolism of homocysteine.29 It is well known that hyperhomocysteinemia can be corrected by increasing folic acid and pyridoxine intake.30–32 Unfortunately, we did not carry out any measurements of pyridoxine or B₁₂ in our study.

A recent study from the Hammersmith Hospital, UK has reported that supplementation of folic acid and vitamin B₁₂ improves vascular endothelial function. This effect is probably mediated through reduction in plasma homocysteine concentrations.33 However, in contrast a report from Addenbrooke's Hospital, UK reported no significant change in arterial stiffness in acute hyperhomocysteinemia induced by an oral methionine load.34 Overall, the association of homocysteine with CAD is still unclear and the American Heart Association has not recommended testing for homocysteine as part of the screening for cardiovascular risk in population-based studies.35

Conclusions: The present study suggests a lack of association between homocysteine and CAD in urban South Indian male diabetic and nondiabetic subjects. The results, however, must be interpreted with caution due to several limitations of this study. Firstly, the sample size was very small. Secondly, we did not adjust for other cardiovascular risk factors, again due to the small sample size. Moreover, while the groups with CAD were diagnosed based on coronary angiography, the diagnosis in patients without CAD was made on clinical grounds and resting ECG findings. One cannot exclude the possibility that had coronary angiography been performed, some of these individuals might have had CAD. This could not, however, be done because of logistic, ethical and socio-economic reasons. Finally, only a fasting homocysteine (basal) sample was estimated. If an oral methionine load was given, the results may well have been different. Prospective studies are clearly needed to study the role of homocysteine in the development of CAD in native Indians.

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References


